Experimental anterior spine fusion using bovine bone morphogenetic protein: a study in rabbits

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Abstract: We developed an experimental model to study the merit of bovine bone morphogenetic protein (bBMP) injection into the intervertebral disc to induce anterior interbody fusion. A total of 24 rabbits, divided into three groups of 8 animals each, were used. One hundred and fifty µg of partially purified bBMP was employed in the first group and 10 µg of bBMP in the second group. In the control group, a sham operation was performed. The animals were followed radiographically at weekly intervals and animals were killed 3, 6, and 12 weeks postoperatively. After sacrifice, a mechanical and histologic evaluation of fusion was performed. Results of radiographic and histologic evaluation showed bBMP formation, which had resulted in the bridging of adjacent endplates, in the 150-µg group. In the 10-µg group, new bone formation was less extensive. In the control group, intradiscal bone formation was seen in only 1 animal. Range of motion measurements on flexion/extension films showed significantly decreased motion in segments that were fused with 150-µg of BMP. This study demonstrated the utility of an experimental model which allowed investigation of how anterior spine fusion may be further studied. Intradiscal injection of BMP could ultimately play a role in the development of minimally invasive techniques for anterior spinal fusion.

Key words: bone morphogenetic protein, anterior spine fusion, spondylodesis

Introduction

Anterior interbody fusion was first described by Csapner 1932,3 and is a well established operative procedure for the treatment of degenerative, traumatic, infectious, or tumors disorders of the spine.6,10,16,20 However, the surgical technique is demanding and is often associated with non-unions. A review of 18 original papers by Salis-Soglio revealed a mean pseudarthrosis rate of 37.5%.25

Methods for improving clinical outcomes included the use of various graft materials. As such, autologous bone from the iliac crest is widely accepted to achieve solid fusion.3 Others have employed allogeneic bone grafting with good results.3,16,20 However, donor site morbidity6,17,32 and a slight but troubling risk of introducing a variety of viruses, including those that cause AIDS or hepatitis,9 are the known disadvantages of the use of these materials. In the search for alternative techniques of producing spinal fusions, osteoinductive substances such as bone morphogenetic proteins have been employed, mainly in posterior or posterolateral fusions.28,30

Several authors analyzed fusion rates by comparing osteoinductive carrier materials containing BMP with autologous or allogeneic material, and demineralized bone matrix. Boden et al.7 performed intertransverse lumbar fusions in rabbits using bovine-derived osteoinductive proteins, and demonstrated fusion rates superior to those seen with autologous bone material or demineralized bone matrix. Similarly, Lovell et al.10 reported higher posterior fusion rates in the thoracic spine in dogs with the use of bovine BMP than with autologous bone grafts. Muschler et al.33 noted similar improvements of posterolateral fusions in dogs with the application of BMP-2. Finally, there is a great body of literature on the use of recombinant human BMP-2 (rhBMP-2) in various animal models, demonstrating its ability to induce posterior and posterolateral fusions.13,22,26,27,28

In this study, we investigated whether BMPs could be used for the enhancement of anterior fusions when injected into the intradiscal space, to provide the basis for the development of minimally invasive surgical techniques. To date, only a limited number of studies have supported the merit of BMP use in anterior

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fusion. However, if feasible, the successful use of BMPs in anterior fusion could offer an array of minimally invasive procedures with improved fusion rates.

Material and methods

Twenty-four 10-week-old New Zealand white rabbits were divided into three groups, of 8 animals each, according to the amount of bovine BMP (bBMP) received: group 1, 150μg; group 2, 10μg; the third group was a control and received a sham operation. The animals were anesthetized by subcutaneous injection of fentanyl, 0.2 mg/kg, and flunisone, 10 mg/kg and were prepared and draped in standard surgical fashion. After dissection of the retroperitoneal space through a midline abdominal incision, the L3/4 disc was identified and punctured, using a 1.5-mm trocar, and the nucleus pulposus was removed. Special care was taken not to damage the adjacent endplates.

In the first group, 150μg bBMP was used for implantation. In the second group, 10μg bBMP was employed. The bBMP and associated insoluble non-collagenous proteins (bBMP/NCP) were prepared from bovine cortical bone by previously described chemical extraction methods and partial purification by differential precipitation. Purified bBMP/NCP (supplied by Dr. M.R. Urist, of the Bone Research Laboratory, University of California Los Angeles, Los Angeles, California) was used as received. The bBMP/NCP used in this study was bioassayed in a mouse thigh muscle assay and found to induce bone formation in over 97% of 36 implants.

After the application of the BMP, the defect in the annulus fibrosus was closed with fibrin glue. In the control group, the defect was closed with fibrin glue alone. A metal marker was implanted to identify the surgical site for radiographic identification during follow-up. The wounds were closed by layers, using resorbable material. Buprenorphinum (0.06 mg/kg/12 h s.c.) was given for pain relief for 3 postoperative days. The animals were monitored for gross neurologic deficits.

During the follow-up period, lateral radiographs of the lumbar spine, with a constant film-focus distance of 1 m, were obtained immediately after the operation and thereafter at weekly intervals until the animals were killed. The animals were evaluated 3, 6, and 12 weeks postoperatively by the killing of two animals in each group 3 weeks postoperatively and three animals in each group at postoperative weeks 6 and 12. Animals were killed with an overdose of pentobarbital (120 mg/kg i.v.), and the lumbar spines were retrieved. Soft tissues were removed and lateral radiographs were taken. Specimens were also examined manually to assess new bone formation and range of motion of the operated segment. Radiographs were evaluated for new bone formation and changes in the height of the intervertebral disc space. The radiographic appearance of calcifications was classified as discrete calcifications and structural calcifications.

For mechanical testing, the caudal vertebral body was mounted and a constant load provided by a weight of 250 g was applied to the cranial vertebral body. Lateral radiographs of specimens were obtained both in flexion and extension for measuring the angle between lines drawn along the posterior wall of the adjacent vertebral bodies, as the intervertebral range of motion. For histologic examination, spinal specimens were fixed in formalin and cut in half in the sagittal plane. The specimens were then dehydrated in graded alcohol and embedded in paraffin. Serial longitudinal 5-μm sections were obtained and stained with hematoxylin and eosin, elastica van Gieson, and Masson-Goldner stains. Specimens were analyzed for new bone formation at the implantation site. An analysis of variance (ANOVA) test was used to test for statistical significance to analyze the range of motion in the operated segments, based on eight animals per group. A P level less than 0.05 was considered statistically significant.

Results

Inspection and palpation of the retrieved spinal segments revealed a hard mass anterior to the operated disc in all eight group-one animals who received 150μg of bBMP. In four of these eight animals, there was no detectable segmental motion. One of these four animals was killed 6 weeks after operation and the remaining three were killed 12 weeks postoperatively. In the group-two animals, which had received 10μg of bBMP, a palpable bony mass anterior to the operated disc was seen in only four of eight animals. Among these four, one animal was killed 3 weeks postoperatively and another animal, 6 weeks postoperatively. The remaining two of these four animals in the 10-μg group with a palpable bony mass were killed 12 weeks postoperatively. Segmental motion was present in all of the animals in the 10-μg group. In the control group (sham-operated) only one of the eight animals had a palpable bony mass at the operated level without fusion 12 weeks postoperatively.

In group one (150-μg bBMP), four animals showed radiographic evidence of bony bridges (Fig. 1A). In the remaining four animals, discrete calcifications were radiographically evident. In addition to these calcifications, two of three animals killed 12 weeks postoperatively had bony bridges in the area of the posterior longitudinal ligament. However, there was no radio-
in Table 1. Injection of 150μg bBMP resulted in a significantly decreased range of motion when compared with findings in both the 10-μg bBMP and the control group (P < 0.05; analysis based on eight animals per group). There was no significant difference between the 10-μg bBMP group and the control group.

Histologic examination revealed new bone formation in the intradiscal space between the two adjacent endplates and under the anterior longitudinal ligament in all animals in the 150-μg bBMP group at all three postoperative intervals. As shown in Fig. 3, this new bone bridged the anterior cortical rims of the vertebral body. Thin bony bridges were also seen between the posterior cortical rims of adjacent vertebral bodies. There was no formation of cartilage or bone within the intervertebral disc. The epiphyses of the vertebral bodies were included in the process of new bone formation.

In the 10-μg bBMP group, histologic findings were similar to those in the 150-μg bBMP group. However, the newly formed bone failed to bridge the anterior cortical rims of the adjacent vertebral bodies of the operated segment (Fig. 4). In two animals in this group, (killed 3 weeks postoperatively) and in one animal (killed 6 weeks postoperatively) there was no histologic evidence of new bone formation.

In the sham-operated control group, there was little evidence of new bone formation. Histologic analysis of the specimens in the control group showed only minimal formation of cartilage ventral to the operated disc and a small bony spur in one of eight animals. Narrowing of the disc space was noted in six of eight animals in this group, but there were no major histologic changes when segments were compared with adjacent non-operated segments.

**Discussion**

Numerous experimental studies have demonstrated the merit of chemically extracted bovine BMPs and human recombinant BMPs for posterolateral spinal fusions. Sheehan et al. achieved a faster and larger fusion mass than in control animals by adding
rhBMP-2 to autologous bone material. Also, Hollinger et al.\textsuperscript{17} found larger fusion masses and greater contact areas with the transverse processes in posterolateral rhBMP-2 fusions. Sandhu et al.\textsuperscript{26} did not find any relation between the dosage (58μg versus 2300μg) of rhBMP-2 and the fusion rate. In contrast, David et al.\textsuperscript{7} reported a dose-related effect of rhBMP-2, comparing 54μg and 860μg in a fusion model in dogs. Finally, Schimandle et al.\textsuperscript{27} were able to achieve a posterolateral lumbar fusion in a wider area more rapidly with rhBMP-2 than with autologous bone material. Implantation of BMPs in a well vascularized host bed provided by the posterior muscle groups of the spine is common to all of these studies. New bone formation was reliably induced, suggesting that large numbers of target cells responsive to BMPs are provided by the posterior soft tissues. These cells were shown to be essential to trigger the cascade of new bone formation.\textsuperscript{16,23,24}
In anterior spinal instrumented fusions, the host bed is less vascularized than in posterolateral fusions, and BMPs are implanted in tissues, such as the intervertebral disc, that have a lower population of responsive target cells. This hypothesis is supported by Kato, who has also used BMP to induce anterior interbody fusions in rabbits. In contrast to our study, they used chymopapain for chemical necrosis and noted a higher frequency of calcifications and bone formation in their experimental model. It appeared that chemical necrosis may have made the host bed more suitable for invasion by mesenchymal cells responsive to BMPs. We noted new bone formation primarily between the anterior cortical rims of the vertebral bodies. In comparison to Kato, we did not see additional ossifications in the nutrient pulposus. Our study clearly showed that induction of new bone formation was dose-dependent. Fusions at the anterior cortical rims occurred exclusively in the 150-µg group, which may explain the larger reductions in the height of the intervertebral space seen in this group. Histologic evidence of new bone formation was present as early as in the third postoperative week, resembling the well-established time sequence of BMP-induced osteogenesis. The BMPs used in our investigation are known to exhibit a chemoattractive effect on chondrogenic and osteogenic progenitor cells, resulting in their migration to the site of BMP application, followed by the formation of enchondral cartilage and differentiation into bone within 4 weeks.

Several explanations for our finding of calcifications and new bone formation in the two control animals appear possible. The transperitoneal approach to the disc may have caused soft tissue trauma; a known element in the formation of ectopic calcifications. Therefore, these calcifications in the control group may represent a nonspecific effect. The mechanical testing of segmental range of motion by loading fixed spinal segments in flexion and extension was previously used with nonoperated rabbit lumbar spines, showing that maximal flexion/extension range of motion can be achieved without damaging the specimen. This simple testing setting was suitable for this investigation of maximal range of motion.

Although the bridging of two adjacent spinal segments by new bone formation between the anterior cortical rims is far from an ideal interbody fusion, this study demonstrates a potential use for BMPs in a poorly vascularized host bed. Although the spinal fusions in this study were not complete (at least not in the time period studied), the lack of complete anterior spinal fusion in this rabbit interbody fusion model does not diminish the finding that bone morphogenetic proteins are clearly capable of exerting their osteoinductive effect on spinal tissues when used in anterior fusion. It seems necessary to further substantiate this statement before BMPs can be used clinically for anterior spinal fusion. Thus, BMPs may play a role in the development of new minimally invasive techniques in the near future.

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